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EFFECTS OF HARVEST DATE AND CURING ON THE COMPOSITION AND PALATABILITY OF PECAN NUTS[1]

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INTRODUCTION

Pecan nuts harvested at relatively early stages of maturity, it has been noticed, are nearly identical in flavor after curing with riper nuts harvested and cured at later dates. Thor and Smith [2] analyzed freshly harvested pecan nuts at intervals from blossoming to maturity and found that most of the constituents of the kernel continued to increase until the nuts were fully mature. Other workers [3 4 5] have reported the analysis of kernels of cured nuts, but no work has been reported to show the effects of curing on kernel composition. Therefore, it was deemed advisable to determine (1) the effect of curing on the composition of nuts; (2) the effects of different curing temperatures on the composition of nuts, particularly of the kernels; and (3) whether nuts harvested at different stages of maturity are affected similarly by the curing process. The results of such a study are reported in this paper.

MATERIALS AND METHODS

Pecan (*Carya illinoensis* K. Koch; syn. *c. pecan* (Marsh.) Engl. and Graebn.) nuts of two standard varieties, Western and Success, growing in the orchard of the United States Department of Agriculture Pecan Field Station, Brownwood, Tex., were selected on the trees for uniformity of size and maturity and tagged on October 1, 1940. Some of these nuts were harvested at each of three later dates for curing and analysis. The first harvest of each variety was made when the nuts were fairly mature. At that time the shucks were green and had just begun to part at the sutures but had not opened enough for the nuts to begin drying. The harvesting of nuts at this stage of development is generally considered an early harvest. The first harvest was made on October 4 for the Western variety and on October 15 for the Success.

The second harvest was made about 1 week after the first and is considered a midseason harvest. At this stage the shucks were green except around the sutures where they had opened. At these edges the shuck tissues had begun to turn brown and dry out. The shucks were still attached to the nuts, although they had opened up sufficiently to allow some drying of the exposed nut shells. The second harvest was

[1] Received for publication December 19, 1942.

[2] THOR, C. J. B., and SMITH, C. L. A PHYSIOLOGICAL STUDY OF SEASONAL CHANGES IN THE COMPOSITION OF THE PECAN DURING FRUIT DEVELOPMENT. Jour. Agr. Res. 50: 97–121, Illus. 1935.

[3] CRANE, H. L., and HARDY, M. B. INTERRELATION BETWEEN CULTURAL TREATMENT OF PECAN TREES, THE SIZE AND DEGREE OF FILLING OF THE NUTS, AND THE COMPOSITION OF THE KERNELS. Jour. Agr. Res. 49: 643–661. 1934.

[4] FINCH, A. H., and VAN HORN, C. W. THE PHYSIOLOGY AND CONTROL OF PECAN NUT FILLING AND MATURITY. Ariz. Agr. Expt. Sta. Tech. Bul. 62: 421–472. 1936.

[5] WOODROOF, J. G., and WOODROOF, N. C. THE DEVELOPMENT OF THE PECAN NUT (HICORIA PECAN) FROM FLOWER TO MATURITY. Jour. Agr. Res. 34: 1049–1063. 1927.

made on October 11 for the Western variety and on October 23 for the Success.

The third harvest was made when the nuts were fully tree-ripened and almost ready to drop-out of the shuck. In picking the nuts at this stage it was difficult to prevent separation of the shucks from the nuts. The Western nuts in this stage were harvested October 24; those of the Success were harvested November 14. The Success nuts in this late harvest were more mature than those of the Western variety harvested on October 24.

At each harvest date 450 nuts were collected and divided at random into 6 lots. Any poorly filled or defective nuts were discarded, and 67 nuts were finally used in each lot for curing and for sampling for analysis.

The six different lots for each harvest date and for each variety were treated as follows:

Lot 1. Separated into shucks, shells, and kernels and sampled immediately.
Lot 2. Shucks removed from nuts, and both cured at room temperature.
Lot 3. Nuts cured at room temperature without removing the shucks.
Lot 4. Shucks removed and nuts cured in refrigerator at 0° to 2° C.
Lot 5. Shucks removed and nuts cured in an air oven at 35° C.
Lot 6. Shucks removed and nuts cured in an air oven at 50° C.

Each lot of nuts subjected to curing was weighed at frequent intervals and was considered to be cured upon reaching approximately constant weight. After being cured, lots 2 and 3 were separated into shucks, shells, and kernels, and samples from each tissue were taken for analysis. The nuts of other lots were separated into shells and kernels which were sampled for analysis. The procedure was the same for nuts of each variety and for each harvest date, except that, in the case of the late-harvested nuts of lot 2, only the shells and kernels were analyzed.

The kernels were analyzed for reducing sugars; total sugars; acid-hydrolyzable polysaccharides; organic nitrogen; either extract, or oil; and dry matter. The shucks and the shells were analyzed for all these constituents except ether extract. Thor and Smith [6] found that freshly harvested shells contained no appreciable amount of hexose sugars or oil and that shucks contained no oil, but it was not known whether sugars would develop in the shells during the curing process.

The methods of sampling and analysis were the same as those used by Thor and Smith,[6] except that sugar analyses were made according to the method of Schaffer and Hartmann.[7]

The analytical data were calculated as milligrams or grams of constituents per kernel, shuck, or shell, based on the average dry weight of these tissues per nut on the date of sampling. By this method the actual changes in nut composition are apparent regardless of the percentage composition.

[6] See footnote 2.

[7] SCHAFFER, P. A., and HARTMANN, A. F. THE IODOMETRIC DETERMINATION OF COPPER AND ITS USE IN SUGAR ANALYSIS. II. METHODS FOR THE DETERMINATION OF REDUCING SUGARS IN BLOOD, URINE, MILK, AND OTHER SOLUTIONS. Jour. Biol. Chem. 45: 365-390. 1921.

EFFECTS OF DATE OF HARVEST AND CURING ON VARIOUS CONSTITUENTS

The analytical data are summarized in tables 1 and 2. The amounts of reducing sugars in kernels of both varieties were extremely small, as shown in the tables, and are not discussed because the differences are within the limits of experimental error in sampling and analysis. Only traces of reducing and total sugars were present in the shells, and these data are omitted from the tables.

TABLE 1.—*Constituents per kernel, shuck, and shell of Western pecan nuts harvested on different dates and cured under different conditions*

Harvest date and lot	Curing temperature and conditions	Part of nut	Reducing sugars	Total sugars	Acid-hydrolyzable polysaccharides	Organic nitrogen	Ether extract	Dry matter
			Milligrams	*Milligrams*	*Milligrams*	*Milligrams*	*Grams*	*Grams*
October 4, 1941:								
Lot 1	Sampled at harvest	Kernel	9.0	27.4	200.0	70.7	2.68	4.09
		Shuck	299.7	393.0	305.8	22.0	.....	2.91
		Shell	.....	.....	557.8	6.9	.....	2.74
Lot 2	Room temperature, shucks removed.	Kernel	10.0	86.0	144.3	70.6	2.92	3.88
		Shuck	86.8	136.6	356.2	21.0	.....	2.44
		Shell	.....	.....	568.0	6.1	.....	2.71
Lot 3	Room temperature, shucks attached.	Kernel	4.0	130.4	142.8	68.1	2.84	3.85
		Shuck	63.9	88.3	387.0	21.3	.....	2.44
		Shell	.....	.....	497.8	5.6	.....	2.75
Lot 4	0°–2° C., shucks removed.	Kernel	4.6	59.9	154.9	71.2	3.02	4.12
		Shell	.....	.....	538.7	6.6	.....	2.79
Lot 5	35° C., shucks removed	Kernel	8.7	99.0	149.0	71.3	3.06	4.10
		Shell	.....	.....	597.1	6.6	.....	2.90
Lot 6	50° C., shucks removed	Kernel	8.4	57.5	129.2	67.8	3.04	3.99
		Shell	.....	.....	542.6	7.1	.....	2.49
October 11, 1941:								
Lot 1	Sampled at harvest	Kernel	11.5	49.9	223.6	75.0	3.31	4.49
		Shuck	333.0	487.3	310.0	23.1	.....	2.84
		Shell	.....	.....	530.4	7.6	.....	2.78
Lot 2	Room temperature, shucks removed.	Kernel	12.5	99.8	168.2	71.4	3.32	4.38
		Shuck	134.0	240.3	410.3	21.3	.....	2.75
		Shell	.....	.....	508.3	7.4	.....	2.89
Lot 3	Room temperature, shucks attached.	Kernel	12.7	105.0	172.6	72.8	3.33	4.36
		Shuck	95.0	176.0	388.0	20.8	.....	2.61
		Shell	.....	.....	531.0	7.1	.....	2.74
Lot 4	0°–2° C., shucks removed.	Kernel	10.7	70.5	146.1	72.8	3.10	4.07
		Shell	.....	.....	472.7	5.6	.....	2.55
Lot 5	35° C., shucks removed	Kernel	4.8	81.7	154.2	70.8	3.36	4.32
		Shell	.....	.....	546.9	6.5	.....	2.75
Lot 6	50° C., shucks removed	Kernel	7.2	73.2	170.6	70.4	3.42	4.42
		Shell	.....	.....	605.9	8.1	.....	2.92
October 24, 1941:								
Lot 1	Sampled at harvest	Kernel	10.5	106.2	212.8	71.4	3.30	4.30
		Shuck	313.0	409.0	319.6	22.7	.....	2.96
		Shell	.....	.....	544.4	8.1	.....	2.87
Lot 2	Room temperature, shucks removed.	Kernel	13.0	113.0	222.9	75.8	3.45	4.54
		Shell	.....	.....	581.3	7.5	.....	2.83
Lot 3	Room temperature, shucks attached.	Kernel	2.9	111.6	197.5	64.7	3.36	4.43
		Shuck	59.1	77.6	395.7	21.1	.....	2.57
		Shell	.....	.....	533.9	7.8	.....	2.80
Lot 4	0°–2° C., shucks removed.	Kernel	6.7	91.0	204.3	81.6	3.37	4.51
		Shell	.....	.....	509.5	6.4	.....	2.70
Lot 5	35° C., shucks removed	Kernel	7.6	108.1	198.8	81.6	3.44	4.56
		Shell	.....	.....	593.6	7.8	.....	2.91
Lot 6	50° C., shucks removed	Kernel	11.2	103.6	154.3	75.5	3.47	4.58
		Shell	.....	.....	552.0	8.5	.....	2.91

TABLE 2.—*Constituents per kernel, shuck, and shell of Success pecan nuts harvested on different dates and cured under different conditions*

Harvest date and lot	Curing temperature and conditions	Part of nut	Reducing sugars	Total sugars	Acid-hydrolyzable polysaccharides	Organic nitrogen	Ether extract	Dry matter
			Milligrams	*Milligrams*	*Milligrams*	*Milligrams*	*Grams*	*Grams*
Oct. 15, 1941:								
Lot 1	Sampled at harvest	Kernel	14.6	50.4	342.5	80.9	3.64	5.06
		Shuck	278.0	423.2	279.3	21.0	.....	2.66
		Shell	.....	.....	809.9	10.7	.....	3.99
Lot 2	Room temperature, shucks removed.	Kernel	10.7	155.0	163.2	82.5	3.59	4.80
		Shuck	45.0	60.9	364.5	21.0	.....	2.25
		Shell	.....	.....	784.7	9.9	.....	4.03
Lot 3	Room temperature, shucks attached.	Kernel	8.5	191.7	170.6	81.3	3.78	4.99
		Shuck	20.3	32.5	372.0	21.0	.....	2.14
		Shell	.....	.....	664.0	9.8	.....	3.76
Lot 4	0°–2° C., shucks removed	Kernel	7.6	109.6	217.1	87.1	3.77	4.98
		Shell	.....	.....	771.2	11.1	.....	4.14
Lot 5	35° C., shucks removed	Kernel	11.5	114.8	170.3	82.4	3.61	4.88
		Shell	.....	.....	737.6	11.0	.....	4.08
Lot 6	50° C., shucks removed	Kernel	14.7	108.0	176.6	82.7	3.59	4.84
		Shell	.....	.....	884.4	13.1	.....	4.07
Oct. 23, 1941:								
Lot 1	Sampled at harvest	Kernel	12.1	65.1	291.5	86.1	3.89	5.35
		Shuck	294.0	432.4	287.3	22.0	.....	2.76
		Shell	.....	.....	912.7	11.7	.....	4.16
Lot 2	Room temperature, shucks removed.	Kernel	9.3	149.2	202.6	101.8	4.05	5.39
		Shuck	48.1	84.7	393.8	24.0	.....	2.36
		Shell	.....	.....	821.8	11.3	.....	4.44
Lot 3	Room temperature, shucks attached.	Kernel	6.2	176.1	222.0	87.9	3.90	5.20
		Shuck	26.5	33.0	371.4	22.0	.....	2.19
		Shell	.....	.....	744.1	10.8	.....	3.90
Lot 4	0°–2° C., shucks removed	Kernel	4.6	104.3	204.3	96.9	3.94	5.24
		Shell	.....	.....	713.4	10.8	.....	3.92
Lot 5	35° C., shucks removed	Kernel	10.9	137.8	180.7	95.0	3.95	5.25
		Shell	.....	.....	786.0	12.0	.....	4.17
Lot 6	50° C., shucks removed	Kernel	12.9	118.3	223.6	81.6	3.90	5.20
		Shell	.....	.....	864.4	12.1	.....	4.16
Nov. 14, 1941:								
Lot 1	Sampled at harvest	Kernel	16.2	167.4	227.7	97.4	3.90	5.41
		Shuck	202.5	254.6	421.3	26.0	.....	2.58
		Shell	.....	.....	846.4	11.3	.....	4.26
Lot 2	Room temperature, shucks removed.	Kernel	14.5	164.6	218.9	86.8	3.91	5.20
		Shell	.....	.....	776.5	12.2	.....	3.90
Lot 3	Room temperature, shucks attached.	Kernel	4.1	155.7	218.9	89.4	3.88	5.14
		Shuck	88.4	102.7	339.6	23.9	.....	2.21
		Shell	.....	.....	730.7	9.7	.....	3.85
Lot 4	0°–2° C., shucks removed	Kernel	8.5	149.7	220.6	90.1	3.83	5.12
		Shell	.....	.....	733.2	10.3	.....	3.89
Lot 5	35° C., shucks removed	Kernel	42.0	163.5	208.4	93.4	3.82	5.16
		Shell	.....	.....	874.7	12.4	.....	4.23
Lot 6	50° C., shucks removed	Kernel	21.9	131.2	220.4	92.0	3.91	5.26
		Shell	.....	.....	851.9	11.6	.....	4.17

REDUCING SUGARS

In shucks of freshly harvested nuts the amount of reducing sugars was relatively large. It increased slightly in the Western variety with the later harvest dates. In shucks of the Success variety the amount of reducing sugars increased slightly between the first and second harvest dates, but it decreased considerably between the second and third dates of harvest.

In the shucks of both varieties the reducing sugars decreased greatly during the curing period at room temperature, but in shucks of the Success variety harvested early and at midseason the decrease was greater than in those of the Western variety at comparable harvest dates. Also there was a greater decrease of reducing sugars in shucks of both varieties attached to the nuts during the curing process than in those that had been detached.

In shucks of late-harvested Success nuts the loss of reducing sugars during the curing process was less than in those of nuts harvested earlier.

TOTAL SUGARS

In kernels of both varieties total sugars had increased at each successive harvest date, there being nearly four times as much sugar per kernel in Western nuts harvested October 24 as in those harvested October 4, and more than three times as much in Success kernels harvested November 14 as in those harvested October 15. The sugars in the kernel were almost all nonreducing.

There was a great increase in total sugars during the curing period in the kernels of nuts of both varieties harvested early and at midseason. In Western kernels harvested late (October 24) there was a slight increase in total sugars under all curing temperatures used except 0° to 2° and 50° C. In Success kernels harvested late (November 14) total sugars decreased somewhat during the curing process, the greatest decrease occurring in kernels cured at 50°.

The greatest increase of total sugars in kernels of both varieties was found in nuts of the first (early) and second (midseason) harvest dates that were cured at room temperature with the shucks attached. The least increase was in kernels of nuts cured at 0° to 2° and 50° C.

In the shucks, in contrast to the kernels, the greater part of the total sugars is reducing sugars, and as would be expected the changes in total sugars were parallel to those in the reducing sugars.

ACID-HYDROLYZABLE POLYSACCHARIDES

The acid-hydrolyzable polysaccharides of the kernels decreased considerably during the curing period in both varieties of nuts from the early and midseason harvests. There was only a slight decrease in this constituent during curing of Success kernels of the late harvest, but in late-harvested Western nuts it decreased considerably in the kernels cured at 50° C.

There was a slight decrease in acid-hydrolyzable polysaccharides of shells from early to late harvest in the Western variety. In shells of the Success variety there was a slight increase toward the later harvest dates, but there was no consistent change in this constituent in shells of either variety during curing. The acid-hydrolyzable polysaccharide content per shell of Success was much higher than that of Western, owing mainly to the larger size of the Success shells.

In shucks of both varieties the acid-hydrolyzable polysaccharide content was relatively high. It increased slightly in Western and markedly in Success during the ripening of the nuts on the trees and increased considerably when the shucks were cured at room temperature except in those of late-harvested Success nuts.

ORGANIC NITROGEN

There was no consistent change in the organic nitrogen content of kernels in either the Western or the Success variety during the curing process. Nitrogen increased appreciably in Success kernels with the later dates of harvest, but not in Western kernels.

The organic nitrogen content of the shucks was low in both varieties harvested early and at midseason and both contained almost the same amount per shuck, although the Western shucks contained more dry matter. There was not much change in this constituent in shucks during the curing process, but the shucks of the Success variety harvested late in the season (November 14) contained considerably more nitrogen than those harvested earlier.

The shells contained very small amounts of organic nitrogen, and there was no consistent change during the curing of the nuts. The higher amounts of organic nitrogen per shell in the Success variety were due largely to the fact that the Success shells were larger than those of the Western.

ETHER EXTRACT, OR OIL

In both varieties the amount of ether extract, or oil, per kernel increased from the early to the midseason harvest dates, after which it remained almost constant. In the Western variety there was an appreciable increase in the amount of ether extract per kernel during the curing process in nuts harvested early (October 4), but no appreciable change in this constituent in kernels of nuts harvested at the two later dates. There was no appreciable change in the amount of ether extract of Success kernels under any of the curing conditions. The amount of ether extract per kernel was greater in the Success variety than in the Western, because the Success kernels were larger.

DRY MATTER

There was an increase in the average amount of dry matter of kernels of nuts from the early to the midseason harvest dates, probably due largely to increases in oil content. The average increase in dry matter per kernel in the Western variety from October 4 (early harvest) to October 11 (midseason harvest) was 8.4 percent, or slightly more than 1 percent each day, whereas the average increase from October 11 to 24 was 3.4 percent. In the Success variety the average increase in dry matter was 7 percent from October 15 to 23, after which there was no further increase. These averages are based on the dry-matter content of all nuts in the six lots at each harvest and include the cured lots as well as those sampled immediately after harvest. In both varieties the increases are statistically highly significant.

The dry matter was slightly lower in kernels of most of the lots of both varieties after curing.

The amount of dry matter per shuck was greater at each harvest date in the Western variety than in the Success. In the shucks of both varieties the dry matter decreased slightly during curing at room temperature. In shells the dry-matter differences probably can be accounted for by variations in the original weight and size of the shells.

EFFECTS OF CURING TEMPERATURE ON FLAVOR, COLOR, AND TEXTURE OF KERNELS

The kernels of nuts cured at 50° C. were very brittle, and the flavor was similar to that of partly roasted kernels. The shells were

also very brittle. The color of the kernels was lighter than that of those cured at room temperature or at 35°. The moisture in the kernels averaged slightly more than 1 percent, which was approximately one-third of that of kernels cured at room temperature. These facts apply to all nuts cured at 50° regardless of the date of harvest.

The kernels of nuts cured at 0° to 2° C. were much lighter in color than those cured at 50°, but they lacked sweetness and appeared to be tough and flabby in texture. These effects were less pronounced in nuts of the later harvest dates than in those harvested early. The moisture content was only a little higher than that of kernels cured at room temperature.

Kernels of nuts cured at room temperature were normal for the variety in flavor, color, and texture, whereas those from nuts cured at 35° C. were normal in color and flavor but were somewhat brittle.

DISCUSSION

Certain interrelations may be noted in the changes occurring in the tissues of pecan nuts under the experimental conditions described.

SUGARS AND ACID-HYDROLYZABLE POLYSACCHARIDES

During the curing period at room temperature, the kernels of nuts of the early and midseason harvest dates with shucks attached increased more in total sugars (consisting chiefly of nonreducing sugars) than did the kernels of nuts with the shucks removed. This difference may have resulted from the translocation of some of the reducing sugars lost from the shucks during the same period and their transformation to nonreducing sugars in the kernels. However, a considerable part of the increase of total sugars in the kernels probably resulted from the conversion of other substances into sugars, since the kernels of nuts with the shucks removed also showed a considerable increase in total sugars under similar curing conditions.

The decrease in the acid-hydrolyzable polysaccharides of the kernels was sufficient to account for the increase in total sugars during curing, but the increase in sugars was not consistently proportional to the decrease in acid-hydrolyzable polysaccharides. Neither of these constituents changed materially during the curing process in nuts of the late harvest, except in those cured at 50° C.; and in the Success variety the acid-hydrolyzable polysaccharides in the kernels of freshly harvested nuts decreased with the later dates of harvest, while total sugars increased during the same period.

The relatively small increases of total sugars in kernels of nuts during curing at 0° to 2° and 50° C. indicate that these extremes of temperature were less suitable for sugar formation than room temperature.

In nuts cured with the shucks attached, the decrease in the reducing sugars of the shucks was greater than the increase of total sugars in the kernels. Moreover, none of the reducing sugars from detached shucks could have been translocated to kernels, yet these shucks lost a considerable amount of reducing sugars. It is possible that part of the reducing sugars was transformed into acid-hydrolyzable polysaccharides, which generally increased appreciably in the shucks during the curing period.

The slight changes in the acid-hydrolyzable polysaccharides of the shells of both varieties tend to confirm the finding of Thor and Smith [8] that shell formation is practically complete in the pecan nut before much development of the kernel takes place.

OTHER CONSTITUENTS

There were changes in other constituents of the nut tissues from harvest to harvest and during the curing process under various conditions. That nitrogen increased appreciably in Success kernels with the later dates of harvest, but not in Western kernels, may indicate that the proteins are later in forming in the Success than in the Western variety, since most of the organic nitrogen in pecan kernels is contained in the proteins.

Increases in dry matter of kernels from early to midseason harvest dates were probably due largely to increases in oil content; in both varieties these increases were statistically highly significant. The loss of dry matter in the kernels during curing may have been due to loss of carbon from respiration and to slight variations in the original size of the kernels.

PALATABILITY

The effects of the highest and the lowest curing temperatures (0° to 2° and 50° C.) on the palatability of the kernels were distinctly unfavorable. Only kernels cured at room temperature were normal for the variety in flavor, color, and texture, whereas kernels of nuts cured at 35° were normal in flavor and color but were slightly brittle.

SUMMARY AND CONCLUSIONS

A study has been made of the effects of harvest dates and curing on the composition of pecan nuts of two varieties, Western and Success.

Some important changes in composition occurred in kernels and shucks of nuts from early to midseason or late harvest dates and during the curing periods. The amount of total sugars increased greatly in kernels as the date of harvest advanced; it also increased considerably during the curing periods in kernels of nuts of the early and midseason harvests, the greatest increase occurring in kernels of nuts cured at room temperature with the shucks attached. The least increase of total sugars during curing occurred in kernels of nuts cured at 0° to 2° and at 50° C. Acid-hydrolyzable polysaccharides decreased in the kernels during curing, and some of these substances may have been converted into sugars. The shucks lost a considerable amount of reducing sugars during the curing period, while an increase in acid-hydrolyzable polysaccharides occurred at the same time. This indicates that at least a part of the reducing sugars was converted into acid-hydrolyzable polysaccharides.

Acid-hydrolyzable polysaccharides decreased slightly in the shells of the Western variety with the later dates of harvest, but the reverse was true in shells of Success nuts. There was no consistent change in this constituent during the curing process in shells of either variety.

[8] THOR, C. J. B., and SMITH, C. L. A PHYSIOLOGICAL STUDY OF THE PREFILLING PERIOD OF FRUIT DEVELOPMENT IN THE PECAN. Jour. Agr. Res. 58: 905-910, illus. 1939.

There was no appreciable change in organic nitrogen of the tissues during curing, or for different harvest dates, except that it increased in Success kernels with the later dates of harvest. This may indicate that proteins continue to be formed at later stages of development in Success kernels than in Western kernels, since most of the organic nitrogen of the kernels is contained in the proteins.

Ether extract, or oil, increased in kernels of both varieties from the early to the midseason harvest dates, after which it remained almost constant. There was a slight increase of oil in early harvested Western kernels during the curing period, but the oil content remained almost constant in all others.

There was a statistically significant average increase in dry matter of kernels of nuts of both varieties harvested at midseason as compared with those harvested early, probably due largely to increases in the oil content. These average increases amounted to 8.4 percent and 7 percent in the Western and Success varieties, respectively.

Kernels of nuts cured at 50° C. were very brittle, and the flavor was impaired in that they tasted somewhat like partly roasted kernels. This was true of all nuts cured at 50°, regardless of date of harvest. Kernels of nuts cured at 0° to 2° lacked sweetness, the texture was flabby and tough, and the color was lighter than that of kernels of nuts cured at the higher temperatures; but these effects were less pronounced in nuts of the later harvest dates than in those harvested early. Kernels of nuts cured at room temperature appeared to be normal in flavor, color, and texture regardless of the date of harvest, while kernels of nuts cured at 35° were normal in flavor and color but were somewhat brittle.

A DISEASE OF GLOXINIA CAUSED BY PHYTOPHTHORA CRYPTOGEA[1]

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INTRODUCTION

Gloxinia (*Sinningia speciosa* Benth. and Hook.) is a highly prized floricultural plant, in California primarily grown in pots for the florist trade. Plants are grown either from seeds or corms (commercially termed "tubers"), usually under glass though occasionally under lath in protected coastal areas. The culture of gloxinia represents an appreciable capital investment.

During the summer of 1938 a disease caused by *Phytophthora cryptogea* Pethyb. and Laff. affecting the corm, stem, and leaves of the plant was observed on potted and bench-grown plants in several large commercial glasshouses in Capitola and San Francisco, Calif. The disease was of economic importance, causing a considerable loss of plants.

It is the purpose of this paper to discuss the symptoms of the disease, the causal organism, and its host range.

REVIEW OF LITERATURE

Few reports appear in the literature of a gloxinia disease which resembles the condition found by the authors. With the exception of a preliminary report by two of the authors (*8*)[2] the reports that do exist are all from Europe, none originating on this continent. Apparently the first record of a leaf and stem rot of gloxinia caused by a species of *Phytophthora* is a report from the Netherlands by Van Poeteren (*10*) in 1926. Landgraf (*4*) briefly describes a corm, stem, and leaf rot in Germany in 1932; no specific organism is reported to be responsible, *Botrytis*, *Moniliopsis*, *Phytophthora*, and *Pythium* all being associated with the disease. Mehlisch (*6*) in 1935 wrote a short description of a soft rot of gloxinia to which he applied the name "Falscher Mehltau," or false mildew; the causal organism was referred to as *Phytophthora speciosa*, but was not described. In 1936 (*2*) *P. parasitica* Dastur was said to be the agent responsible for a stem and leaf rot of gloxinia in England and Wales. Pape (*9*) in 1937 reported a stem and leaf rot in Germany and made several suggestions for the control of the disease as it occurs in the glasshouse; an unidentified *Phytophthora* sp. was said to be associated with the disease. Williams et al. (*17*) report that *P. cactorum* (Leb. and Cohn) Schroet., isolated from *Erica hiemalis* Hort. in England, was capable of infecting gloxinia stems upon inoculation. The most recent contribution is that of Schmidt (*13*) who reiterates the remark of Mehlisch that gloxinia mildew is caused by *P. speciosa*.

[1] Received for publication March 9, 1943. Joint contribution from the Division of Plant Pathology, California Agricultural Experiment Station (Riverside and Berkeley), and the Department of Botany, Missouri Agricultural Experiment Station (Journal Series paper No. 888).

[2] Italic numbers in parentheses refer to Literature Cited, p. 412.

Some of the disease symptoms on gloxinia due to *Phytophthora* may resemble those arising from other causes. Meurs (*7*) states that *Pythium debaryanum* Hesse is responsible for a root rot of gloxinia in the Netherlands. Van Poeteren (*11, 12*) reports soft, black, sunken lesions on gloxinia corms received in the Netherlands from Belgium and said to be due to *Cylindrocarpon radicicola* Wr. Marchal (*5*) records *Thielavia basicola* (Berk. and Br.) Zopf from gloxinia corms in Belgium, stating that the fungus caused superficial, black encrustations on affected corms. *Thielaviopsis basicola* (Berk.) Ferraris has been reported from corms in England and Wales (*1*).

SYMPTOMS OF THE DISEASE

The disease affects the corm, stems, and leaves of the plant. The symptoms of the disease are identical on all varieties of gloxinia that have been found infected. In naturally infected plants the leaves are somewhat discolored, water-soaked, dark brown, and quite flaccid (fig. 1). Within 2 to 7 days, under favorable conditions for the development of the disease, infection may progress from the lamina of the leaf to the petiole, causing the death and collapse of the leaf. In badly diseased plants the stems may be attacked; quite often stem infection is noted in the absence of leaf infections. Infected plants at first appear stunted and wilted (fig. 2), later they may become chlorotic, collapsing as infection spreads into the petioles (fig. 3). Diseased stems bear sunken, water-soaked lesions which may be rather narrow and vertically disposed, or may be quite large and encompass the stem.

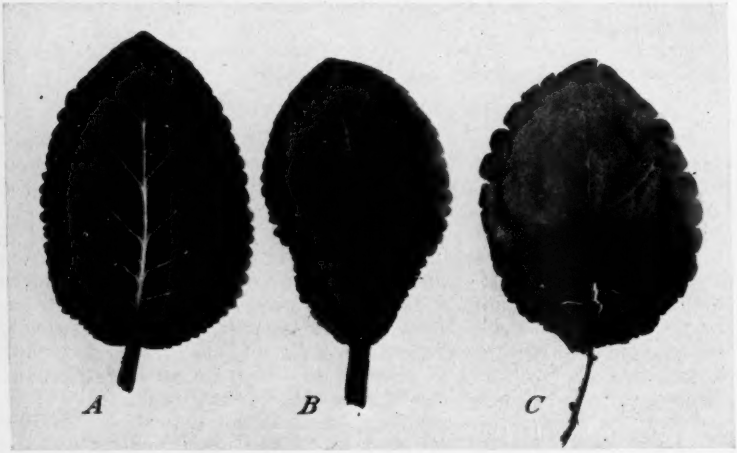

FIGURE 1.—Leaf rot of gloxinia: *A*, Noninoculated control; *B*, *C*, symptoms produced on leaves of gloxinia by *Phytophthora cryptogea* 12 days after inoculation in the glasshouse.

Corm infection may be associated with stem or leaf infection or may occur independently. Infected corms exhibit soft, sunken, surface lesions. Severely diseased corms usually have dark-brown, soft,

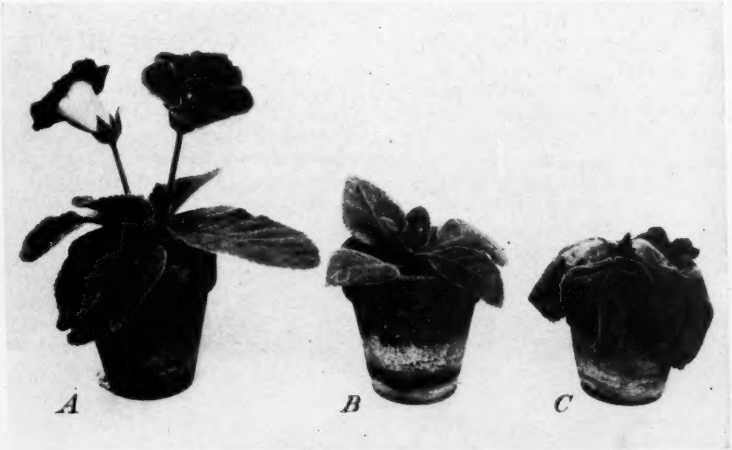

FIGURE 2.—Phytophthora disease of gloxinia: *A*, Noninoculated control; *B*, stunting, and *C*, wilting produced on gloxinia plants by *Phytophthora cryptogea* after inoculation in the glasshouse.

FIGURE 3.—Phytophthora disease of gloxinia: *A*, Noninoculated control; *B*, *C*, chlorosis and rot produced on gloxinia plants by *Phytophthora cryptogea* after inoculation in the glasshouse.

internal necrotic areas, 1 to 8 mm. in diameter, erratically disposed throughout the underground storage organ (fig. 4). These infected regions may or may not be directly associated with the more common surface lesions. The fibrous roots of diseased plants are often infected, particularly those roots that are associated with infected areas on the surface of the corm. Infected roots are darkly discolored and flaccid.

Recently, Ark and Tompkins (*3*) have described a boron-deficiency disease of glasshouse-grown gloxinia in which the leaf lamina shows a brownish-black discoloration and loss of turgidity, eventually causing the plants to die. This disease may sometimes be confused with the one discussed in this paper.

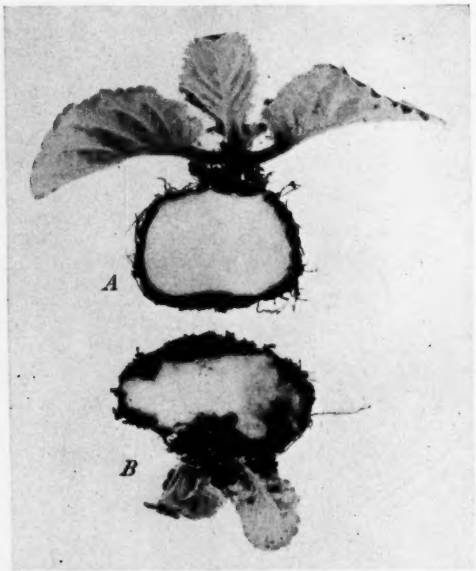

FIGURE 4.—Corm rot of gloxinia: *A*, Noninoculated control; *B*, symptoms produced on gloxinia corms by *Phytophthora cryptogea* 28 days after inoculation in the glasshouse.

THE CAUSAL FUNGUS

Isolations made from diseased leaves, stems, and corms on water or malt-extract agars have consistently yielded a fungus which has been identified as *Phytophthora cryptogea*.

MORPHOLOGY

Reproductive bodies rarely develop on the usual agar media; in old cultures on oatmeal agar, oögonia with a mean diameter of approximately 25μ were occasionally found. Each oögonium contained an oöspore, the mean diameter of which was approximately 22μ. The antheridia were of the persistent, amphigynous type. When hyphae

were transferred to distilled water or nonnutrient solutions, sporangia developed in a few days. They were nonpapillate, ovoid, obpyriform or elongated, straight or slightly curved, with a mean size of approximately 35μ long and 20μ wide. Small spherical to irregular vesicular hyphal swellings developed in large numbers; they were hyaline, thin-walled and usually disposed in dense clusters.

TEMPERATURE RELATIONS

The relation of temperature to growth of the mycelium was determined for four isolates of *Phytophthora cryptogea* from leaves, stem, and corm. The culture tubes used and the procedure followed in this study are those previously described by Tompkins and Gardner (*14*). Each tube used is provided with a glass dam at the open end. Thirteen milliliters of Difco corn-meal agar (pH 6.0) are added to each tube and allowed to cool to a uniform depth with the vessel in a horizontal position; the dam prevents the escape of the melted agar.

The tubes were planted near the dam at the open end with a small square of potato-dextrose agar containing the mycelium of the fungus cut from 5-day-old Petri-dish cultures. Inoculated tubes were left at room temperature for 36 hours, the extent of growth at the close of the period being indicated by a wax pencil mark on the tube; subsequent measurements were made from this point.

Three tubes of each isolate were placed in a horizontal position in controlled temperature chambers at intervals of 3°, from 1° to 34° C. The cultures were incubated for a 96-hour period. The amount of growth in millimeters for the 96-hour period at the various temperatures is presented in table 1.

TABLE 1.—*Mycelial growth of Phytophthora cryptogea on Difco corn-meal agar at various temperatures*

P. cryptogea from corm, leaf, and stem	Growth in millimeters over 96-hour period											
	1° C.	4° C.	7° C.	10° C.	13° C.	16° C.	19° C.	22° C.	25° C.	28° C.	31° C.	34° C.
Corm	6	9	12	18	26	36	46	55	58	47	30	0
Leaf	5	8	13	18	25	36	48	56	58	43	21	0
Do	6	10	13	16	24	35	45	53	55	45	25	0
Stem	7	10	13	17	25	36	47	55	57	46	27	0

The minimum temperature for mycelial growth is below 1° C., the optimum between 22° and 25°, and the maximum between 31° and 34°. These values are in conformity with those given by Tucker (*16*).

PATHOGENICITY

All isolates of the fungus from gloxinia proved pathogenic to healthy gloxinia plants grown from seeds and corms. Inoculum was prepared by growing the fungus on sterilized cracked wheat in 8-inch test tubes. When ready for use, this was added to autoclaved soil contained in 6-inch pots in such a manner as to avoid injuring the root system of the young plants. Sterile cracked wheat was added to pots serving as controls. All pots were heavily watered each day in order to keep the soil very moist. The incubation period ranged from 14 to 28 days

for seedling plants, but all infected plants died within 2 to 6 days after the foliage commenced to wilt. The incubation period for plants from corms was somewhat longer, depending upon the size of the corm planted; the range was usually from 18 to 35 days. Of 435 plants inoculated, none escaped infection, while all the control plants remained healthy. The fungus was reisolated from a representative sample of plants and proved to be identical with the original isolates; when tested in parallel series, the reisolates again proved pathogenic.

Symptoms on artificially infected plants were identical with those on naturally infected plants. Contrary to the report of Pape (*9*) that the blue-flowered varieties of gloxinia are most susceptible to infection by *Phytophthora* sp. than the white varieties, no difference in susceptibility was found in blue, white, red, or variegated varieties when inoculated with *P. cryptogea*. However, this does not exclude the possibility of the existence of varieties resistant to this disease, as only a relatively few varieties were tested.

EXPERIMENTAL HOST RANGE

Various hosts were inoculated by the technique described for the pathogenicity tests. Reisolations were made from all diseased specimens; the reisolate of the fungus from a particular host was then tested by inoculation into healthy plants of that host.

A number of species of the Gesneriaceae were first tested. Approximately 40 plants of each species were inoculated with *Phytophthora cryptogea* from gloxinia. Plants of *Achimines cardinalis* A. Dietr., *A. grandiflora* DC., *A. longiflora* DC., *Aeschynanthus lobbiana* Hook., *A. speciosus* Hook., *Alloplectus schlimii* Planch. and Lind., *Episcia cupreata* Hanst., *Gesneria cardinalis* Lehm., *Isoloma amabile* Mott., *I. hirsutum* Regel, *Naegelia cinnabarina* Lind., *N. multiflora* Hook., *N. zebrina* Regel, *Saintpaulia ionantha* Wendl., *Sinningia speciosa*, and *Streptocarpus kewensis* Hort. proved to be susceptible.

A résumé of natural and artificial infection of certain plants by *Phytophthora cryptogea* as reported by various investigators has been given by Tompkins and Tucker (*15*), who have presented also several new host records for this fungus. No members of the Gesneriaceae are indicated. The fungus has been isolated and identified by the writers more recently from *Prunus amygdalus* Batsch (almond) and *Cotoneaster lacteus* W. W. Smith. Cultures of the species isolated from *Dianthus caryophyllus* L. (carnation) and *Ceanothus prostratus* Benth. were received from Kenneth F. Baker.

Isolations of *Phytophthora cryptogea* from *Calceolaria crenatiflora* Cav. (slipperwort), *Callistephus chinensis* Nees (China-aster), *Celosia argentea* L. var. *cristata* (L.) O. Ktze. (cockscomb), *Godetia grandiflora* Lindl. (godetia), *Iris* sp., *Senecio cruentus* DC. (cineraria), gloxinia, and *Tagetes erecta* L. (African marigold) were used in inoculation experiments to determine the susceptibility of slipperwort, China-aster, cockscomb, *Gerbera jamesonii* Hook. var. *transvaalensis* Hort. (Transvaal daisy), godetia, *Matthiola incana* R. Br. var. *annua* Voss (annual stock), cineraria, and gloxinia to infection. The results are presented in Table 2.

Annual stock, China-aster, Transvaal daisy, and cineraria have previously been reported (*15*) as susceptible to *Phytophthora cryptogea*.

TABLE 2.—*Artificial inoculation of various plants with different isolates of Phytophthora cryptogea*

Host from which isolated	Plant inoculated [1]							
	Annual stock	China-aster	Cineraria	Cockscomb	Gloxinia seedling	Godetia	Slipperwort	Transvaal daisy
African marigold	+	−	+	+	+	−	−	+
China-aster	+	+	+	−	−	−	−	+
Cineraria	+	+	+	+	+	+	+	+
Cockscomb	+	−	+	+	+	−	+	+
Gloxinia:								
Corm	+	+	+	−	+	+	+	+
Stem	+	−	+	+	+	−	−	+
Godetia	+	−	+	−	−	−	+	+
Iris	+	−	+	−	−	−	−	+
Slipperwort	+	−	+	+	+	+	+	+

[1] + = Positive infection; − = no infection.

DISCUSSION

In the absence of any description of *Phytophthora speciosa*, which is supposedly a new species responsible for a rot of gloxinia in Europe, the epithet must be considered a nomen nudum. In England a disease similar to that caused by *P. speciosa* is said to be due to *P. parasitica*. The authors have recovered only *P. cryptogea* from diseased gloxinia; this is believed to be the first report of a *Phytophthora* disease of gloxinia in America, the first record of the occurrence of *P. cryptogea* on this host, and the first report of this species on the leaves of any host.

The causal fungus is able to infect not only gloxinia, but also a number of other species of the Gesneriaceae. The following are all new host records for *Phytophthora cryptogea: Achimines cardinalis, A. grandiflora, A. longiflora, Aeschynanthus lobbiana, A. speciosus, Alloplectus schlimii, Episcia cupreata, Gesneria cardinalis, Isoloma amabile, I. hirsutum, Naegelia cinnabarina, N. multiflora, N. zebrina, Saintpaulia ionantha,* and *Streptocarpus kewensis* Hort.

It is apparent from the results given in table 2 that annual stock, cineraria, and Transvaal daisy are more susceptible to attack by *Phytophthora cryptogea* than China-aster, cockscomb, gloxinia, godetia, and slipperwort. There is also some evidence that certain isolates of *P. cryptogea* may have a wider host range than others; the isolate from cineraria was able to infect annual stock, China-aster, cineraria, cockscomb, gloxinia, godetia, slipperwort, and Transvaal daisy, whereas the isolate from iris was able to infect only annual stock, cineraria, and Transvaal daisy, hosts which were shown to be susceptible to all nine isolates used.

Cineraria, cockscomb, and slipperwort are new hosts for *Phytophthora cryptogea* and so far as the writers are aware have not been previoulsy recorded as such in the literature.

SUMMARY

A corm, stem, and leaf disease of gloxinia is prevalent on the San Francisco Peninsula and in the vicinity of Capitola, Calif.

Symptoms of the disease consist of soft, sunken, surface lesions and

internal dark-brown, soft necrotic areas, 1 to 8 mm. in diameter. Diseased stems bear sunken, water-soaked lesions which may be rather narrow and vertically disposed, or may be quite large and encompass the stem. Infected leaves are water-soaked, dark brown, and flaccid.

The causal organism has been identified as *Phytophthora cryptogea* Pethyb. and Laff.

The minimum temperature for mycelial growth is below 1° C., the optimum between 22° and 25°, and the maximum between 31° and 34°.

Phytophthora cryptogea has been demonstrated to be pathogenic to 9 genera and 15 species of the Gesneriaceae in addition to gloxinia. Cineraria, cockscomb, and slipperwort are newly reported hosts for *Phytophthora cryptogea*.

This is apparently the first record of *Phytophthora cryptogea* on gloxinia, and the first instance of this fungus on leaves of any host.

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RELATION BETWEEN HOT-WATER EXTRACTIVES AND DECAY RESISTANCE OF BLACK LOCUST WOOD[1]

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INTRODUCTION

The heartwood of black locust (*Robinia pseudoacacia* L.) shows considerable variation in durability. Within the commercial range of black locust, farmers commonly refer to at least two kinds of locust in respect to durability, viz, (1) "yellow" locust, characterized by a dark-yellow heartwood, which is reputed to last 50 to 100 years in contact with the soil, and (2) "white" locust, characterized by a gray heartwood, which is reputed to last only 10 to 30 years in contact with the soil. Less frequently, such terms as "green," "red," "gray," and "black" are used by farmers in describing color variations of heartwood that in their experience are associated with differences in durability.

Hirt (*7*)[4] investigated this reputed difference by laboratory culture tests of the wood of two selections of locust from Long Island, N. Y.: Shipmast locust, which is a "yellow" variety; and common locust, which is a "white" variety. One tree of each variety was tested. The study showed that the heartwood of the shipmast locust tree was more resistant to decay than the heartwood of the common locust tree.

Subsequently, heartwood from a large number of trees, representing several different varieties, was tested by the writers. The results, as yet unpublished, indicate definitely that there are significant differences between varieties in the durability of their wood. The tests show also that smaller but nevertheless substantial differences in wood durability exist between trees of different sizes and ages belonging to the same variety, as well as between different positions within the same tree.

These laboratory investigations and field observations indicate that locust strains of superior wood durability may be selected for large-scale planting to replace the unselected locust used at present, provided a simple, rapid method can be developed to test the wood durability of the many varieties under consideration. Studies by different investigators on several tree species having durable wood indicate that resistance of the heartwood to decay is commonly due in large measure to the presence of toxic extractives. It therefore appeared desirable to determine whether a simple extractive test could

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[2] In cooperation with the Forest Products Laboratory, Madison, Wis.
[3] The authors acknowledge with appreciation their indebtedness to W. E. Manis and N. A. Norton for their able assistance with the decay tests.
[4] Italic numbers in parentheses refer to Literature Cited, p. 426.

be used as a practical index of the durability of the wood of the various black locust selections.

Two separate experiments were conducted in the study reported here. The first experiment was a test with extractives from black locust heartwood to determine the effect of various concentrations of the same extractives on the growth rate of an appropriate decay fungus. The second experiment was a test of various black locust trees and strains to determine whether their relative resistance to decay may be ascertained by measuring either the concentration of extractives in the heartwood or the toxicity of the extractives to the decay fungus.

REVIEW OF LITERATURE

Working with 15 woods, including both British and North American species, Ernest (*5*) showed that many of them had water-soluble extractives that were in various degrees toxic to decay fungi. Hawley et al. (*6*) found that the cold-water and hot-water extractives from 10 native species of wood are toxic to the growth of a wood-destroying fungus. They concluded that the generally accepted relative resistance to decay of the woods tested could be largely explained by the toxicity of their water-soluble components. Black locust was one of the woods in which the presence of toxic extractives was demonstrated. A similar finding was reported by Sowder (*12*) and by Cartwright (*4*) for western red cedar (*Thuja plicata* D. Don), and chemists at the Forest Products Laboratory (unpublished data) have isolated several toxic extractives from the heartwood of that species. Moderately toxic water-soluble extractives have even been found in ponderosa pine (*Pinus ponderosa* Dougl.) (*1*), a species with comparatively low decay resistance.

The resistance to decay of various parts of the heartwood of virgin redwood (*Sequoia sempervirens* (Lambert) Endlicher), as shown in laboratory tests, was found by Sherrard and Kurth (*10*) to vary with the distribution of the water-soluble extractives. A similar relationship was noted in a limited preliminary study on two black locust trees by Hopp and Hirt.[5]

MATERIALS AND METHODS

SELECTION OF TREES

Eleven black locust trees, cut on Long Island, N. Y., were used in this study. Two of the trees belonged to the shipmast selection (*8*); four represented an undescribed variant designated as Flowerfield locust; and the remaining five were of a heterogeneous nature, grouped under the general name "common locust." As the shipmast and Flowerfield variants bear practically no seed, the individual trees of each of these two groups were probably closely related genetically. The common locust trees were seed-bearing types and came from separate stands; hence, several variants were probably represented. The trees were all approximately 10 inches in diameter at breast height.

PREPARATION OF TEST BLOCKS

Materials for testing were taken from 1-foot sections of the trunks at heights of 7 to 8 feet. From the heartwood of these sections were sawed, in radial order, groups of five small blocks, consisting of four

[5] HOPP, H., and HIRT, R. R. EXPERIMENTS ON THE RELATIVE DECAY RESISTANCE OF BLACK LOCUST SELECTIONS. U. S. Soil Conserv. Serv., Division of Hillculture Research, in cooperation with the N. Y. State Col. Forestry, Syracuse Univ. 1939. [Unpublished report.]

decay specimens[6] and one reference specimen for computing the initial oven-dry weights of the decay specimens (fig. 1). The test blocks were ½ by 2 by ¼ inches in the radial, tangential, and longitudinal directions of the wood, respectively. In addition to the small blocks, one larger block was taken at each radial position for the extractive analyses. The blocks were numbered consecutively according to their respective radial position, inward from the sapwood to the pith. Each position, which included the width of a test specimen and saw kerf, occupied approximately 0.65 inch on a radius.

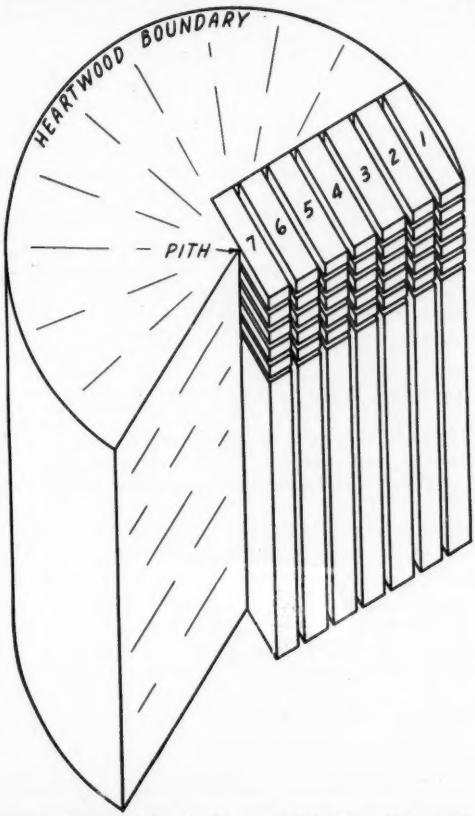

FIGURE 1.—Blocks for decay tests (small blocks) and for extractive comparisons (large blocks) in a tree section. All blocks in each vertical series were given the same serial number, beginning with the heartwood boundary as indicated.

The decay tests and the extractive analyses at any particular radial position were thus made on virtually replicate material, since the wood in each group of blocks came from essentially the same place in the same growth rings. Each radius was completely sampled except where suitable material, free from knots or locust-borer damage, was not available.

[6] Only two of the four decay blocks were used in the present study; the other two were used for tests not considered here.

DECAY TESTS

The amount of decay produced in the blocks by the test fungus was measured by the percentage loss in the oven-dry weight of the wood. The blocks were first dried in a special room where the relative humidity was maintained at 30 percent and the temperature at 80° F. The blocks were then weighed and sterilized by heating in an autoclave for 20 minutes at 100° C. Following this, the blocks to be decayed were placed, under aseptic conditions, in 6-ounce wide-mouth bottles, of the French square type, having screw tops from which the paper lining had been removed. Each bottle contained 25 ml. of malt-agar medium (2.5 percent of Trommer's plain diastasic malt extract and 1.5 percent of Bacto-agar in distilled water), which had hardened while the bottles were in a horizontal position. The specimens were laid, one in a bottle, on a 3-mm. V-shaped glass rod resting on the hardened substrate, and the substrate was inoculated immediately with a pure culture of the test fungus. The cultures were then incubated for 4 months at a constant temperature of 80° F. and a relative humidity of 80 percent. At the end of the incubation period the blocks were wiped free of loose surface growth of the fungus, oven-dried at 105° C., and again weighed. The reference blocks, which had been stored in dry containers, were oven-dried and weighed at the same time. In determining weight losses, the initial oven-dry weights of the decayed blocks were computed from their initial air-dry weights and the weight losses on drying of the corresponding undecayed reference blocks.

DETERMINATION OF EXTRACTIVE CONTENT

For the extractive determinations, the large block from each radial position was reduced to sawdust and the proportion of oven-dry wood soluble in hot water was determined in accordance with the standard procedure followed at the Forest Products Laboratory (*3*). The initial oven-dry weight of the extractive samples was computed rather than determined directly, according to the procedure described for the decay specimens. The hot-water extractive rather than the cold-water extractive was measured because of indications in the work by Hopp and Hirt[7] that the former more closely paralleled decay resistance. Each extractive analysis was made in duplicate. The solutions of extractive material were carefully retained for the toxicity tests.

MEASUREMENT OF EXTRACTIVE TOXICITY

Experiment 1

The relative toxicity of the extractives was measured by the linear growth rate of the decay fungus on the standard culture medium (see Decay Tests) containing the extractives. For the first experiment, in which the toxic effect of different concentrations of the same extractives was to be determined, extracts from two samples were used. These samples were obtained from (1) radial position 1 of Flowerfield tree B46–2 and (2) radial position 1 of common tree A54–18, trees that differed markedly in decay resistance. The extractives were tested at seven different concentrations, as listed in the results.

[7] See footnote 5, p. 422.

Since the extract solution contained known amounts of extractive, the desired extractive concentrations in the culture media were obtained by diluting or by evaporating portions of the extract solutions, as required. The solutions were evaporated, in beakers, at 50° C., or diluted to a few milliliters less than the final volume, and the requisite quantities of malt extract and agar were finally added to each preparation. The mixtures were then heated in an autoclave for 20 minutes at 100° to liquefy the agar. Following this, enough hot distilled water was added to the preparations to give exactly the predetermined total amounts of medium required for the desired concentrations of extractives. The resultant hot medium was then mixed by blowing air into it through a pipette, and immediately thereafter was pipetted into special cotton-plugged test tubes, where it was first sterilized by autoclaving or steaming and then allowed to harden. Ten milliliters of medium was placed in each tube. To reduce evaporation losses and attendant changes in the composition of the medium, the tubes were capped with lead foil.

Because of the possibility that autoclaving might tend to alter the extractives more than a milder sterilization treatment, two types of sterilization were tried: (1) Heating for 1 hour at 100° C. on 3 successive days and (2) autoclaving for 15 minutes at 15 pounds, steam pressure. The latter type of sterilization was used throughout the second experiment.

The test tubes in which the growth-rate measurements were made were 2.5 cm. in diameter and 20 cm. long and were modified by a rounded indentation in the wall near the mouth (*9*). The medium was cooled with the tubes in a horizontal position, and the indentation formed a dam that prevented escape of the medium while it was still liquid. The agar strip thus formed provided a substrate about 15 cm. long and of uniform cross section throughout the length of the tube.

The medium was inoculated near the midpoint with a small piece of mycelial mat of the test fungus, and subsequent linear growth in both directions was measured from a mark made on the glass. The temperature in the incubation chamber was approximately 28° C., although some small variation was unavoidably produced by occasional higher outside temperatures. The total linear extension of both advancing margins of the fungus occurring between the fourth and the ninth day following inoculation was taken as the measure of rate of growth, 4 days being allowed for the fungus to become well established on the new substrate. To put the measurements for this experiment on the same time basis as those for the second experiment, the respective rates were computed to a 7-day period.

Experiment 2

In the second experiment, the extractive contents were determined for each radial position of all the trees dealt with, but the toxicity of the extractions was tested only on the Flowerfield and common locust trees, since these selections showed the greatest difference in decay resistance. The toxicity tests of samples from the Flowerfield trees were made in duplicate and of those from the common trees in quadruplicate. The mixtures of extractive and malt agar were prepared for these tests in each case by evaporating all the extractive solution

from a 2-gm. sample of sawdust to a volume of about 20 cc., then adding sufficient malt extract, agar, and distilled water to give a total volume of hot culture medium of 25 ml. The final concentrations of malt extract and agar were, as in the previous media preparations, 2.5 percent and 1.5 percent, respectively, and the concentrations of extractives in the media were between 0.25 and 1.0 percent, which, from the results of the first experiment, were judged to lie in a range most suitable for comparing toxicities as measured by the growth rate of the test fungus. The rest of the procedure in making the toxicity tests was the same as that followed in experiment 1. The total amount of linear extension of the fungus during the 7-day period from the fourth to the eleventh day after inoculation was used as the measure of growth rate.

For both the decay tests and the toxicity tests, a strain of *Fomes rimosus* (Berk.) Cke. was used that had been isolated from a decayed locust fence post.

RESULTS

RELATION BETWEEN THE CONCENTRATION AND TOXICITY OF BLACK LOCUST EXTRACTIVES (EXPERIMENT 1)

The objectives of this experiment were (1) to obtain definite verification of the toxic nature of black locust extractives, (2) to determine what concentrations of extractives would give a suitable range of growth rates for the toxicity tests of the second experiment, and (3) to determine which type of sterilization (autoclaving or steaming) should be used. In addition, quantitative data were obtained on the experimental error attached to such biological measurement of the toxicity of locust extractives.

The results of the experiment are presented graphically in figure 2. It is apparent that the tested concentrations of extractive lower than

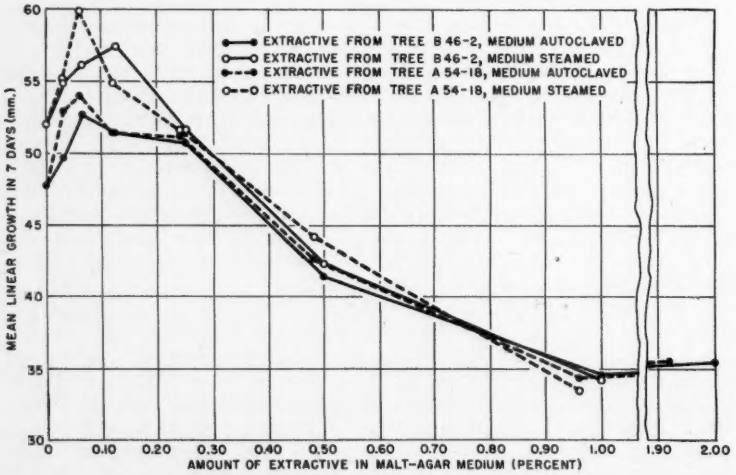

FIGURE 2.—Rate of growth of *Fomes rimosus* on malt-agar medium, autoclaved or steamed, containing various concentrations of extractives from radial position 1 in trees B46–2 and A54–18. (Experiment 1.)

about 0.25 to 0.30 percent stimulated in various degrees rather than retarded growth of the fungus. Such a phenomenon is not uncommon with other decay fungi and with other toxic chemicals in low concentrations (*2*). On the other hand, growth retardation is indicated for all concentrations above about 0.25 to 0.30 percent, and in increasing degree to a concentration of about 1 percent. At concentrations of about 2 percent the growth rate was nearly the same as at about 1 percent, the small difference probably being due to experimental error. This break in the trend was possibly a result of the medium being saturated with toxic ingredients of the extract solutions at concentrations above the 1-percent level. Such a conclusion is further suggested by the presence in increasing amounts of a brown and yellow precipitate in media containing extractives in concentrations of more than 0.24 percent.

The two methods of sterilization did not cause a significant difference in growth rate of *Fomes rimosus* when the extractive concentrations were large enough to cause retardation. When the extractive was absent from the medium or was at a low concentration, in the range of stimulation, steaming was associated with more rapid growth than autoclaving. Consequently, the toxicity tests in experiment 2 were made with concentrations of the extractives in the range of retardation and the media were autoclaved because of the simpler procedure required.

The average variation between results of duplicate cultures indicated that the smallest difference in concentration of extractive in malt agar that can be reliably ascertained by growth-rate measurements is about 0.1 percent.

RELATION OF DECAY RESISTANCE TO THE CONTENT AND TOXICITY OF EXTRACTIVES IN THE WOOD (EXPERIMENT 2)

The weight loss due to decay and the corresponding content and toxicity (in terms of fungus growth rate) of the extractives at each radial position in the trees tested are given in table 1.

The first thing to consider is what variations there were in decay resistance, as indicated by the weight loss in the respective test blocks. The question then arises whether these variations are closely associated either with the amount of extractive in the wood or with the toxicity [8] of the extractives. These relationships can be considered with greater efficiency if the data of table 1 are first compiled by means of covariance analysis. The procedure used in making this analysis is standard (*11*); hence only the final compilations are required here, and these are given in table 2.

A comparison of the original weight-loss variances in table 2 shows that there were a number of statistically significant variations in decay resistance in the material sampled. The variations in decay resistance were associated with the radial position of the blocks in the tree (as shown in table 1, the weight loss of the wood blocks was progressively greater from the outermost to the innermost part of the heartwood), with differences in the several trees of the common locust group but not of the shipmast and Flowerfield selections, and with differences in

[8] In this paper, the expression "toxicity of the extractives" refers to the toxicity of the total amount of extractive obtained from a 2-gm sample of wood. Hence the toxicity (as measured by fungus growth rate) reflects the influence of both the amount and the unit toxicity of the extractives in the wood.

the selections. The mean values in table 1 indicate that the Flowerfield samples showed more over-all resistance to decay than the shipmast and common samples. The extremely large variation among the common locust trees was to be expected, inasmuch as the trees in this group constituted heterogeneous and unselected types whereas the

TABLE 1.—*Relation of decay resistance* [1] *of black locust heartwood to the hot-water extractive content of the wood and to the growth rate of Fomes rimosus on malt-agar medium containing hot-water extractives in proportion to amounts present in the wood*

[Experiment 2]

Selection,[2] tree No., and position of sample (fig. 1)	Loss in weight [3]	Extractive content [4]	Linear growth [5] of fungus in 7 days
Shipmast:			
B6–7:	*Percent*	*Percent*	*Millimeters*
1	2.6	13.07	
2	2.1	12.18	
3	2.8	10.66	
4	3.9	9.83	
5	5.5	8.43	
Mean	3.4	10.83	
B10–8:			
1	1.2	12.54	
2	2.1	10.74	
3	1.3	9.27	
4	3.9	6.84	
5	4.2	4.94	
Mean	2.5	8.87	
Mean	3.0	9.85	
Flowerfield:			
C42–7:			
1	1.3	11.53	38.0
2	.9	11.44	38.0
3	.9	11.64	36.2
4	1.0	9.92	38.2
5	1.3	9.09	39.2
Mean	1.1	10.72	37.9
B46–1:			
1	1.5	11.36	35.5
2	1.8	10.50	35.0
3	1.6	9.62	35.2
4	1.7	10.43	37.2
5	1.7	9.88	36.0
6	2.4	9.30	36.5
Mean	1.8	10.18	35.9
B46–2:			
1	0.8	12.22	33.8
2	1.3	11.06	33.8
3	1.1	10.18	34.2
4	1.1	10.34	36.0
5	1.1	9.84	33.2
6	1.5	9.82	35.5
7	1.4	9.20	34.8
Mean	1.2	10.38	34.5
Flowerfield—Con.			
B46–3:	*Percent*	*Percent*	*Millimeters*
1	1.1	12.15	34.5
2	.8	10.97	35.5
3	.7	10.13	39.0
4	1.0	10.56	35.0
5	1.0	9.89	35.5
6	1.6	8.46	36.0
7	2.3	8.60	32.5
Mean	1.2	10.10	35.4
Mean	1.3	10.32	35.8
Common:			
B53–7:			
1	5.5	5.74	39.9
2	6.3	4.96	45.4
3	8.1	3.10	47.5
Mean	6.6	4.6	44.3
B54–7:			
1	1.1	10.40	40.1
2	1.2	9.60	38.1
3	1.1	7.70	43.2
4	1.8	6.32	43.0
5	2.8	5.87	45.4
Mean	1.6	7.98	42.0
C56–2:			
1	1.3	9.16	37.5
2	2.2	8.08	35.4
Mean	1.7	8.62	36.4
B56–2:			
1	1.2	9.44	37.0
2	1.1	7.85	39.4
3	.9	6.71	40.9
4	2.9	4.76	44.8
Mean	1.5	7.19	40.5
A54–18:			
1	2.8	12.62	39.5
2	4.1	11.36	36.0
3	4.5	10.46	35.6
4	5.3	10.14	39.1
5	3.5	9.74	34.9
6	5.0	9.14	35.4
7	5.3	8.38	34.8
Mean	4.4	10.26	36.5
Mean	3.2	8.17	39.7

[1] Decay resistance is indicated by percentage loss in oven-dry weight of the wood, caused by *Fomes rimosus*.
[2] For convenience, the common locust trees are summarized together. (See p. 416 for varieties used.)
[3] Weight-loss values are means of 2 samples.
[4] Extractive-content values are means of 2 samples.
[5] Growth rates are means of 2 cultures for the Flowerfield samples and 4 cultures for the common samples.

TABLE 2.—*Analysis of covariance of percentage weight loss caused by Fomes rimosus in relation to content of hot-water extractives and toxicity of the extractives*

[Experiment 2: calculated from data of table 1]

Source of variation	Weight loss							
	Original data		Adjusted for extractive content			Adjusted for extractive toxicity		
	Degrees of freedom	Variance	Degrees of freedom	Variance	Correlation between weight loss and extractive content	Degrees of freedom	Variance	Correlation between weight loss and linear growth
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
(A) Among selections: [1]								
Shipmast, common, and Flowerfield	2	23.50**	2	11.56**				
Common and Flowerfield	1	42.17**				1	23.59**	
B) Among trees within selections: [2]								
Shipmast	1	1.76	1	6.76**				
Common	4	18.23**	4	18.10**		4	18.99**	
Flowerfield	3	.59	3	.50**		3	*.61	
(C) Among radial positions within trees: [3]	45	.72	44	.29		36	.39	
Shipmast	8	1.94**	7	.51	−0.88**			
Common	16	.88**	15	.40	−.76**	15	.59**	0.61**
Flowerfield	21	.14**	20	.09*	−.61**	20	.13**	.29
(D) Experimental error (between duplicated samples):								
Shipmast	16	.37						
Common	21	.19						
Flowerfield	25	.04						

[1] Significance of variance determined from variance ratio between A and C.
[2] Significance of variance determined from variance ratio between B and C.
[3] Significance of variance determined from variance ratio between C and D.
* Significant values (5-percent level).
** Highly significant values (1-percent level).

shipmast and Flowerfield variants were each distinct types with relatively uniform characters.

As regards the relation of extractive content and extractive toxicity to these variations in decay resistance, the extractive data in table 1 indicate that both the content and toxicity of the extractives were associated in some degree with the weight loss. These relations within individual trees are expressed by pooled linear correlation coefficients, shown in columns 6 and 9 of table 2. The extractive content is inversely correlated with weight loss, and the correlation coefficients are highly significant for trees of all three selections. Growth rate of the test fungus on the extractive medium was significantly correlated with weight loss within trees of the common locust, but within trees of the Flowerfield locust the correlation was small and not mathematically significant. Toxicity tests were not run, as mentioned previously, on the shipmast locust extractives. From the correlation coefficients obtained for the common and Flowerfield selections, it appears that weight-loss variations among radial positions within the trees were associated more closely with the extractive content than with the measured toxicity of the extractive.

The comparative utility of extractive content and extractive toxicity for indexing the observed weight loss may be further judged by the adjusted variances of columns 5 and 8 in table 2. These data in-

dicate the variation in weight loss among the selections (A), among the trees in each selection (B), and among the radial positions within the trees (C), after such variations as are related to differences in extractive content or toxicity have been accounted for. Any variation that significantly exceeds in magnitude the average variation in weight loss between duplicate tests at each radial position (D, error) can logically be attributed to variable factors other than the extractives.

The variances among radial positions within trees (C), which were highly significant for the original data, are largely accounted for by the extractive content. This is evidenced by the fact that the adjusted variances (C, column 5) are no longer significant in the case of the shipmast locust and common locust trees and barely significant in the case of the Flowerfield locust trees.

The toxicity of the extractives, on the contrary, does not give as promising a result, since the adjusted variances (C, column 8) remain highly significant for the two selections included in this test. The fact that the adjusted variances are somewhat lower than those of the original data and that there is a significant correlation coefficient for the common locust trees indicates that toxicity, as measured by the growth rates in the agar tests, is associated with weight loss, but the relation is not close enough to account for all the significant variation in weight loss among radial positions within the trees.

When the adjusted variances among the trees of the common and Flowerfield selections (B) and between the selections (A) are examined, it is seen that neither extractive content nor extractive toxicity accounts for much, if any, of these sources of variation in weight loss. This is shown by the fact that the adjusted variances among trees and among selections are but slightly or not at all smaller than the corresponding variances calculated from the original data. Moreover, in all cases, these adjusted variances are significantly greater than both the experimental error and the adjusted variance within trees.

The indications from these results, therefore, are that the proportion of hot-water extractives in the wood accounts for almost all the significant variations in weight loss within individual trees, but, on the same basis, accounts very little for differences in weight loss among different selections or among trees within selections. This result might be interpreted as an indication that the toxic composition of the extractives at different radial positions within individual trees was comparatively uniform but that among different trees and selections it was not. While the toxicity of the extractives, as measured by fungus growth rate, might theoretically be expected to index decay resistance better than extractive concentration alone, the results indicate that the opposite actually occurred; nevertheless the two extractive measures were related rather closely.

An interesting side light on the preceding results was the relation between the color of the wood and the extractive content. In general, the greater the amount of yellow in the color of the sawdust sample, the higher was the percentage of hot-water extractive. For example, samples with a light greenish-yellow color by daylight had an average extractive content of 13.30 percent, whereas the percentage in samples with a light chocolate-brown color averaged only 8.38 percent. By ultraviolet light, samples with a deep lemon-yellow color averaged 11.20 percent in extractives, whereas those fluorescing a light straw yellow averaged 8.90 percent. Intermediate colors were roughly asso-

ciated with intermediate extractive contents. These color relations, although not consistent enough to rely on as a basis for estimation of extractive content in the case of individual samples, are quite in line with the widespread belief in the greater durability of locust heartwood that is yellow in color.

CONCLUSIONS AND SUMMARY

The widely recognized superior decay resistance of black locust heartwood appears to be imparted in large measure by certain toxic chemical components. The typical high specific gravity of black locust may contribute to the general high level of decay resistance, but tests not reported here showed that this factor is little if at all related to differences in the decay resistance within this species itself. The present study shows that the hot-water extractives were not only toxic to decay fungi at concentrations considerably lower than those naturally occurring in the wood but that within individual trees they may occur in amounts and with degrees of toxicity roughly proportional to the decay resistance.

On the basis of the tests reported here with *Fomes rimosus*, it appears that the amount of hot-water extractives in the wood accounts to a large degree for the decay resistance of heartwood at different radial positions in the cross section of individual trees, but in only small measure for the differences in decay resistance among different trees or different selections of black locust. As a result of these findings it is suggested that the toxic composition of the extractive is comparatively uniform within individual trees but not among different trees.

A similar relation was generally found between decay resistance and the toxicity of the hot-water extractives as measured by the growth rate of *Fomes rimosus* on malt-agar medium containing the respective hot-water extractives in amounts proportional to the amounts present in the wood. Although theoretically a better test, the toxicity of the extracts did not index decay resistance within individual trees as accurately as did total extractive content.

The failure of the extractive toxicity to index decay resistance at least as well as the amount of extractives may be accounted for partly by the fact that the error in determining extractive content by measuring fungus rate was about three times as great as that in determining extractive content by direct weighing. Thus, even though the extractive toxicity was fully as closely related to decay resistance as extractive content, it would require a larger number of replicate toxicity tests to indicate that such was the case. It is also quite possible, particularly as regards the larger differences in decay resistance among trees and between selections, (1) that the full toxic effect of the extractives was in some manner precluded by failure of the standard method of extraction to obtain all of the toxic material in the wood, (2) that some change occurred in the original toxic material as a result of the extraction process or subsequent treatment of the extractions (this possibility was indicated by precipitates that formed in the culture medium), or (3) that certain of the extractives were sufficiently toxic to effectively retard those physiological processes of the fungus necessary to the digestion of such a complex substance as wood but not to inhibit development of the fungus greatly on a readily utilizable medium such as malt extract. That is to say, on such diverse sub-

strates as malt agar and wood, the manner in which the toxicants are effective conceivably may be basically different.

This study indicates that, with the procedures followed, the extractive content and its toxicity as measured by its effect on fungus growth in artificial culture are inadequate for a precise evaluation of the decay resistance of black locust selections involving different trees or selections. However, since a definite relation apparently exists between these factors within individual trees, it is suggested that further efforts in this direction might be profitable, possibly including tests of water extractions made without high temperatures and, in addition, extractions with nonaqueous solvents.

As a supplementary observation it was noted that the amount of yellow color in the wood, after conversion into sawdust, was roughly in the same order as the total extractive content, but the relation was not consistent enough to use as a basis of estimation in the case of individual samples. However, it may be significant that this observation is in line with the greater durability commonly ascribed to "yellow" locust.

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